

### CLAIM AMENDMENTS

1. (Currently Amended) A method for detecting a biotinidase deficiency and one or more disorders selected from: an aminoacidopathy, a fatty acid oxidation disorder and an organic acid disorder, metabolic disorder in an individual, comprising:

(a) contacting a sample comprising:

(i) biotinidase and

(ii) one or more amino acids or carnitines ~~metabolic analytes~~,

with biocytin and one or more protease inhibitors to produce a reaction admixture, under conditions wherein said biotinidase is capable of acting on said biocytin, to generate at least one product, the product selected from biotin and lysine, and wherein one or more protease inhibitors are present;

(b) contacting said reaction admixture with a reagent that inhibits the ability of said biotinidase to act on biocytin, wherein said one or more amino acids or carnitines ~~metabolic analytes~~ and said at least one product are soluble in said reagent; to produce a test sample and

(c) determining the ~~presence or~~ amount of said one or more amino acids or carnitines ~~metabolic analytes~~ and determining the amount of said at least one product contained in said test sample using mass spectrometry,

wherein a determined ~~presence or~~ amount of said one or more amino acids or carnitines ~~metabolic analytes~~ correlates with presence or absence of said aminoacidopathy, fatty acid oxidation disorder or organic acid disorder and a determined amount of said at least one product correlates with presence or absence of said biotinidase deficiency ~~metabolic disorder~~.

2. (Original) The method of claim 1, wherein said sample is a body fluid sample.

3. (Original) The method of claim 2, wherein said body fluid sample is blood.

4. (Original) The method of claim 1, wherein said sample is dried.

5. (Original) The method of claim 1, wherein said individual is a human suspected of having a metabolic disorder.

6. (Original) The method of claim 1, wherein said individual is a neonate.

7. (Original) The method of claim 1, wherein said individual is a newborn.

8. (Original) The method of claim 1, wherein said individual is a child.

9. (Original) The method of claim 1, wherein said individual is an adult.

10. (Original) The method of claim 1, wherein said metabolic disorder is an inborn error of metabolism.

11. (Original) The method of claim 1, wherein said metabolic disorder is an acquired metabolic disorder.

12. - 15. (Canceled)

16. (Original) The method of claim 1, wherein said metabolic analyte is one or more amino acids.

17. (Original) The method of claim 1, wherein said metabolic analyte is an acylcarnitine or plurality of acylcarnitines.

18. (Original) The method of claim 1, wherein step (a) further comprises contacting said sample with one or more reference substrates.

19. (Original) The method of claim 1, wherein step (b) further comprises contacting said

sample with one or more reference products.

20. (Original) The method of claim 1, wherein step (d) further comprises, prior to determining, adding one or more reference products corresponding to the at least one product.

21. (Original) The method of claim 1, wherein step (d) further comprises, prior to determining, adding one or more reference analytes corresponding to the one or more metabolic analytes contained in said sample.

22. (Original) The method of claim 1, wherein said reaction admixture is aqueous.

23. (Original) The method of claim 22, wherein said reagent is non-aqueous.

24. (Original) The method of claim 23, wherein said reagent comprises an organic solvent.

25. (Original) The method of claim 23, wherein said reagent comprises an alcohol.

26. (Original) The method of claim 25, wherein said reagent is methanol.

27. (Original) The method of claim 1, wherein said mass spectrometry is tandem mass spectrometry.

28. (Previously Presented) The method of claim 1, wherein one of said protease inhibitors is a protease cocktail.

29. (Original) The method of claim 1, wherein one of said protease inhibitors is PEPSTATIN.

30. - 39. (Canceled)

40. (New) A multiplex assay for two or more metabolic indicators in a biological sample from an individual, comprising:

(a) contacting the sample with one or more substrates for one or more metabolically indicative enzymes and with one or more protease inhibitors to produce a reaction admixture, the one or more metabolically indicative enzymes characterized in that increased or decreased activity of the one or more metabolically indicative enzymes in the sample compared to a normal reference activity of the one or more metabolically indicative enzymes is associated with one or more metabolic disorders selected from an aminoacidopathy, a fatty acid oxidation disorder and an organic acid disorder;

(b) reacting the reaction admixture under conditions wherein the one or more metabolically indicative enzymes is capable of acting on the one or more substrates to generate at least one product which is a first metabolic indicator to be assayed;

(c) contacting the reaction admixture with a chaotrope that inhibits the ability of the one or more metabolically indicative enzymes to act on the one or more substrates, wherein the at least one product and a second metabolic indicator to be assayed are soluble in the chaotrope, to produce a test sample, wherein the second metabolic indicator is selected from an amino acid and a carnitine, the second metabolic indicator characterized in that increased or decreased level of the second metabolic indicator in the sample compared to a normal reference level of the second metabolic indicator is associated with one or more metabolic disorders selected from an aminoacidopathy, a fatty acid oxidation disorder and an organic acid disorder; and

(d) determining the amount of the first and second metabolic indicators in the test sample using mass spectrometry.